pH back to 7.4. Further, we explore the effects of pH on channel selectivity and protein-protein interaction of VDAC with dimeric tubulin that is known to block VDAC pore with nanomolar efficiency. To address structural rearrangements upon gating, we also use magic angle spinning NMR to study conformational changes in recombinant human VDAC1 as a function of pH ranging from 3 to 11. Under these conditions we observe reversible changes in chemical shifts upon changing the pH from 7 to 4. These observations support functional results of VDAC voltage-gating at different pH and complement the data with site-specific information about residues affected by pH changes. The mechanism of VDAC gating and its relevance to in vivo situation are discussed.

217-Pos Board B17

Membrane Lipid Composition Regulates Tubulin-VDAC Interaction

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Accumulating evidence suggests that lipids play an important role in mitochondrial function, dynamics, and morphology and in the permeabilization of the mitochondria outer membrane (MOM) that initiates apoptosis. Recently we have found that dimeric αβ-tubulin, a subunit of microtubules, regulates mitochondrial respiration by directly blocking the VDAC pore (Rostovtseva et al., PNAS 2008). Here, we show that the mechanism of tubulin-VDAC interaction is complex and greatly depends on membrane lipid composition. The on-rate of tubulin-VDAC binding varies up to 100-fold depending on the particular lipid used for bilayer formation but is independent of the charge of lipid headgroups, and the presence of cholesterol. The off-rate of tubulin-VDAC binding does not depend on lipid content. The gramicidin A (gA) channel was used to probe the effect of tubulin on lipid bilayer mechanics. We found that 30 nM of tubulin increased gA channel lifetime 10-fold in DOPE bilayers but did not affect it in DOPC. Tubulin effects on gA were significantly reduced in low salt, suggesting a hydrophobic interaction. Using confocal fluorescence microscopy we observed tubulin binding to the membranes of giant unilamellar vesicles (GUVs) made from DOPC and DOPC/DOPE directly. We found that adsorption of the fluorescently labeled dimeric tubulin (Tubulin-HiLyte488) on the GUV membranes requires the presence of DOPE. We did not observe tubulin binding to GUVs made from pure DOPC. We propose that prior to tubulin's characteristic blockage of VDAC by permeation of tubulin's C-terminal tail into the channel lumen, there is an additional, non rate-limiting step whereby tubulin first binds to the membrane. Our findings suggest a new regulatory role of mitochondrial lipids in control of MOM permeability and hence, mitochondrial respiration, through tuning of VDAC sensitivity to blockage by tubulin.

218-Pos Board B18

Tubulin-VDAC Interaction: Salt Dependence of Conductance and Reversal Potential

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The recently reported blocking of mitochondrial voltage-dependent anion channel (VDAC) by tubulin [1] has raised new questions about the role of tubulin C terminal tails (CTT) in the low conductance states exhibited by VDAC channel. The voltage sensitive reversible channel closure observed suggests that tubulin is more than an enhancer of VDAC gating. However, there is no conclusive evidence about the nature of the channel interaction with tubulin tails. Experiments have also revealed that VDAC-tubulin interaction is also influenced by the lipid composition of the membrane hosting the channel. While the duration of tubulin induced closure seems to be independent of salt concentration, the opposite is true with VDAC open time between successive blockages. The change in conductance between open and close states is also concentration dependent. By performing single channel conductance measurements as well as reversal potential measurements (in open and closed states) we analyze the importance of electrostatic interactions between VDAC ionizable residues and the CTT negative charge in the binding of tubulin. The anion selectivity of VDAC in its open state is turned into cation selectivity under tubulin blockage. The recently published 3D structure of VDAC [2] seems fully compatible with the partial penetration of alpha or beta tubulin tails into the channel lumen.

[1] T. K. Rostovtseva et al. PNAS, 105 (2008) 18746.
[2] S. Hiller et al. Trends Biochem. Sci., 35 (2010) 514.

219-Pos Board B19

Reduced Tyrosine Nitration of VDAC and Decreased Apoptosis by Mitochondria-Directed Therapy After Cardiac Ischemia Reperfusion in Isolated Hearts

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Superoxide ($O_2^{\bullet-}$) produced during cardiac ischemia-reperfusion (IR) injury reacts with nitric oxide to form peroxynitrite ($ONOO^-$). $ONOO^-$ induces protein tyrosine nitration (tyr^N) that causes protein structural alteration and dysfunction. The mitochondrial voltage-dependent anion channel (VDAC) plays an important role in regulating the metabolic and energetic functions of mitochondria and contributes to mitochondrial-mediated apoptosis. It is not known if VDAC is nitrated by $ONOO^-$ during IR or how this modification might compromise cardiac function after IR. Because of the importance of VDAC modification, we hypothesized that the clinically used anti-anginal drug ranolazine (RAN), which also reduces cardiac IR injury, does so via a mitochondrial mechanism, i.e., in part by decreasing VDAC tyr^N. To test this, isolated guinea pig hearts were perfused with KR buffer for 40 min (time control, TC), or for 30 min of ischemia plus 10 min of reperfusion, with or without 10 μM RAN infused before ischemia. Mitochondria were isolated at the end of each treatment. VDAC tyr^N was determined by IP with anti-nitrotyrosine antibody (NTab), followed by Western blotting (WB) with anti-VDAC antibody. The effect of RAN on VDAC tyr^N was also examined. Cytochrome *c* release was checked as the marker for apoptosis. We found that enhanced VDAC tyr^N was increased by 108% after IR vs. TC and cytochrome *c* was higher in the cytosol after IR than after TC. RAN treatment decreased VDAC tyr^N by 31%, while decreasing cytochrome *c* release by 38%, compared to IR. These results indicate that VDAC tyr^N and a concomitant increase in cytochrome *c* release occur during IR injury, and importantly, that cardioprotection by RAN occurs in part by reducing VDAC tyr^N, which may impede activation of apoptotic pathways during IR injury.

220-Pos Board B20

A Mitochondrial ATP-Sensitive Potassium Channel from the ROMK Family

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Mitochondrial potassium modulates mitochondrial bioenergetics, and the existence of resident potassium channels in the mitochondrial inner membrane has been amply validated, yet the pore-forming subunits of these channels remain unidentified. We therefore undertook an in-depth proteomic analysis of the mitochondria employing repeated fractionation at the organellar, protein, and peptide levels. Briefly, density-purified inner membranes were extracted with 1% lauryl maltoside, and fractionated by sucrose gradient centrifugation. Each fraction was digested with trypsin and subjected to strong-cation exchange HPLC prior to reversed-phase LC-coupled tandem mass spectrometry. 964 proteins were identified, of which 684 were classified as mitochondrial in UniProtKB and/or MitoCarta databases. From the inner membrane fraction, the ROMK (renal outer-medullary potassium) channel, was identified by 6 spectra matching two overlapping peptides. Matches were statistically validated at >95%. Subsequent bioinformatic analysis using TargetP and PSORT detected a mitochondrial localization sequence near the N-terminus of ROMK. Mitochondrial localization was confirmed in neonatal rat ventricular myocytes (NRVM) transiently transfected with truncation mutants of human ROMK isoforms fused with green fluorescence protein (GFP) or V5-tag at the C-terminus of the channel. Imaging by 2-photon microscopy showed that predicted targeting presequences confer colocalization of GFP with tetramethylrhodamine methyl ester (TMRM) staining of the mitochondria. Furthermore, to determine whether a ROMK isoform might mediate mitochondrial potassium uptake, we measured K^+-dependent swelling in rat heart mitochondria. In preliminary studies, cromakalim-induced mitochondrial swelling of liver mitochondria was abrogated by Tertiapin-Q, a high-affinity ROMK channel toxin, with half-maximal inhibition in the picomolar range. Reverse-Transcription/Polymerase Chain Reaction (RT-PCR) identified 3 isoforms (ROMK1, ROMK2 &ROMK6) in the adult rat hearts and NRVMs. The findings support an isoform of ROMK as a candidate for the pore-forming subunit of mitoK_{ATP}.

221-Pos Board B21

MAC Function Triggers a Bax/Bak Dependent Bystander Effect

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Collateral spread of apoptosis to nearby cells is referred to as the bystander effect - a process that is integral to tissue homeostasis and a challenge in anticancer therapies. In many systems, apoptosis relies on permeabilization of the mitochondrial outer membrane to factors like cytochrome c and Smac/DIABLO. This permeabilization occurs via formation of a mitochondrial apoptosis-induced channel, MAC, and was mimicked here by single-cell microinjection of cytochrome c in Xenopus embryos. Waves of apoptosis were observed in vivo from the injected to the neighboring cells. This finding indicates that a death signal generated downstream of cytochrome c release diffused to neighboring cells and ultimately killed the animals. The role of MAC in bystander effects was then assessed in mouse embryonic fibroblasts that did or did not express its main components Bax and/or Bak. Exogenous expression of GFP-Bax triggered permeabilization of the outer membrane